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Characterization and Identification of Alfalfa and Red Clover Dietary Supplements Using a PCR-Based Method

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The use of herbal remedies is very popular in the United States, with >80 million people buying plant-derived preparations that are often highly degraded or potentially contaminated with nonefficacious plant material. A method utilizing DNA-based markers to identify highly fragmented or powdered plant material sold as botanicals in dietary supplements has been developed. By incorporating and streamlining a repair reaction that utilized fill-in and ligation reactions before the PCR steps, it was possible to amplify highly degraded or sheared DNA isolated from powdered plant material removed from over-the-counter capsules. The primers for the internal transcribed spacer (ITS) region of nuclear ribosomal DNA generate a PCR fragment compatible with the sizes of the repaired DNA. Moreover, a large data set in Genbank facilitated subsequent analysis. This method is a relatively rapid and simple system to facilitate the authentication, as well as the monitoring, of the purity of botanicals in dietary supplements, even those that are improperly dried or stored.

KEYWORDS: PCR amplification; flavonoids; repair reaction; alfalfa; red clover; dietary supplements

INTRODUCTION

According to the World Health Organization, ∼4 billion people worldwide use herbal remedies for primary health care. Many of them are in developing countries, but in the developed world, more and more people have turned to the use of herbal remedies in addition to pharmaceuticals for health enhancement (*1*). In the United States alone, >80 million people use some type of herbal or botanical dietary supplement. However, many of them may not be effective, either because they are degraded or because they are contaminated with nonefficacious plant material. The Dietary Supplement Health Education Act of 1994 mandates the development of good manufacturing practices (GMPs) as one approach to this problem.

Botanicals in dietary supplements are sold both as extracts and as dried plant material, the latter usually consisting of leaves, roots, or flowers. Traditionally, dried and fragmented plant material sold in capsules or pills as botanicals in dietary supplements has been identified to the genus level and sometimes to the species level on the basis of morphological characters, that is, leaf or stem trichomes, or various histological features, that is, distinctive cell types. However, these methods are frequently inaccurate, especially for plant material that is either ground to a very fine powder, field-collected and contaminated with other plants, or highly oxidized or mechanically degraded due to drying or storage under unfavorable conditions.

We sought to develop methods based on Polymerase Chain Reaction (PCR) of isolated DNA from samples of botanicals because DNA is the richest and most unambiguous source of genetic variability. DNA also allows for the precise identification of species because, unlike morphological, anatomical, and phytochemical traits, it is not influenced by environmental conditions; for example, nitrate levels affect hypericin production in St. John's wort (*2*) and soil mineral nutrient supply influences ginsenoside production in American ginseng (*3*). The methods whereby the commercial botanicals are prepared vary from manufacturer to manufacturer and also can affect the composition or concentrations of the compounds under study. Molecular standards for chromatography may also be difficult or too expensive to obtain, especially for rare chemicals or for those that have not been previously characterized.

Various molecular biology techniques, including PCR-based methods, have been used for identifying species ranging from prokaryotes to higher eukaryotes. The success of PCR-based species identification rests on the quality of the DNA isolated from the biological specimens. Consequently, numerous methods have been described to isolate DNA from poorly preserved herbarium material and mummified animal tissue (see ref *4*). One of the most useful of these is the repair reaction described

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by Pusch et al. (*5*) for prehistoric or ancient DNA. Such DNA is frequently not PCR-amplifiable due to either contamination, a low copy number for the desired DNA segment, or severely fragmented DNA. We have modified this repair reaction to isolate PCR-amplifiable DNA from powdered plant material that was removed from capsules of herbal supplements, namely, alfalfa and red clover, and have also incorporated the use of the internal transcribed spacer (ITS) region in the PCR amplification. Although alfalfa is not a commonly used herbal supplement (*6*), red clover is a source of isoflavones, particularly the phytoestrogens genistein and daidzein (*7*), and is thus used as an alternative to estrogen replacement therapy.

MATERIALS AND METHODS

Preparation of Plant Extracts. Fresh tissues were collected in liquid nitrogen, and the frozen tissues were ground in liquid nitrogen using a mortar and pestle. Powders were removed from the gelatin capsules and immediately transferred to HPLC-grade methanol (10 mL of solvent/2 g of tissue). The methanol-soluble components were extracted overnight at 4 °C. After filtration (Whatman No. 1), the extracts were concentrated using a vacuum evaporator at low pressure. They were then diluted in 30% methanol and refiltered by passage through a 0.20-μm sterile filter to remove any large particles before the HPLC analysis. All steps were performed as quickly as possible to avoid oxidation of the flavonoids.

HPLC Analysis. The plant extracts were prepared as described above. Because many flavonoids present in legumes, such as genistin (genistein 7-glucoside), luteolin (5,7,3′,4′-tetrahydroxyflavone), kaempferol (5,3,7,4′-tetrahydroxyflavone), quercetin (3,5,7,3′,4′-pentahydroxyflavone), taxifolin (3,5,7,3′,4′-pentahydroxyflavanone), and myricetin (3,5,7,3′,4′,5′-hexahydroxyflavone), elute between 30 and 50% methanol, the extracts were run in a 25−55% methanol gradient. Compounds dissolved in 25% methanol were injected onto a reverse phase HPLC column (Supelco, Supelcosil LC-18-S, 150 × 4.6 mm, 120 Å, 5 μm) equilibrated in buffer A (methanol/water/glacial acetic acid, 25/72.5/2.5, v/v/v) and eluted at 1 mL/min with an increasing linear gradient of methanol to 55% over 50 min. By using a single-wavelength UV detector, the eluate was monitored at 290 nm. To identify the corresponding peaks of flavonoids, extracts were co-injected with 1 μg of a standard flavonoid. Peaks corresponding to flavonoids were identified by retention time. Standard flavonoids (taxifolin, 8.5 min; genistin, 13 min; myricetin, 25.4 min; quercetin, 36.4 min; luteolin, 42 min; and kaempferol, 45.5 min) were purchased from Sigma (St. Louis, MO).

DNA Isolation. DNA was isolated from freshly harvested leaves of alfalfa (*Medicago sativa* cv. Regen SY; *8*) and red clover (*Trifolium pratense* L.) growing in the UCLA greenhouse using a modified CTAB (*9*) procedure (see http://www.caltech/edu/meyerowitz/protocols/CTAB.htm) or with a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA).

Two independent sample sources of capsules containing alfalfa and red clover dried leaf material were purchased from a local pharmacy. To protect the manufacturers' identity, the samples were labeled A and B. DNA was isolated, following the manufacturer's instructions for the DNeasy Plant Mini Kit, from 25−50 mg, as per the manufacturer's instructions, of plant powder removed from the capsules. The amount of the extracted DNA varied between 60 and 150 ng/μL. Precautions were taken to exclude contamination of the powdered leaf material DNA from the DNA isolated from fresh leaves.

Repair Reaction. The DNA samples isolated from the powders were subjected to a two-step repair reaction that consisted first of a fill-in reaction and a ligation reaction (*5*) followed by a phenol/chloroform extraction, although the latter step was eventually eliminated. For the fill-in reaction, 500−1500 ng of DNA was mixed with 2.5 units of *Escherichia coli* exonuclease-free DNA polymerase I (Amersham Pharmacia Biotech, Piscataway, NJ), 5 μL of 10× nick translation buffer, and 0.4 mM dNTPs (TaKaRa, Shiga, Japan), brought up to a total volume of 50 μL with Sigma-Aldrich HPLC water. The reaction was carried out for 17−24 h at 37 °C and terminated with a 20-min incubation at 70 °C.

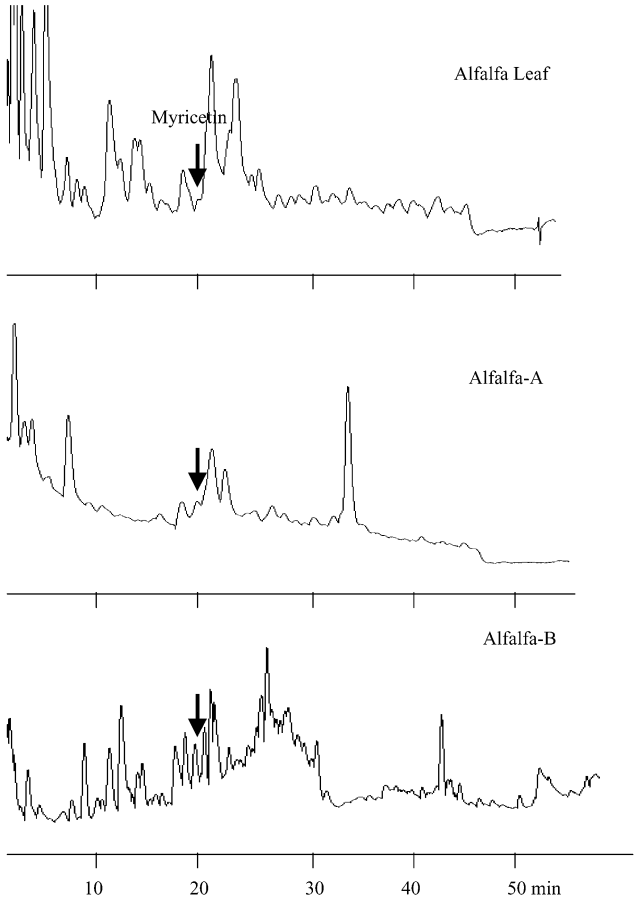

Figure 1. HPLC analysis of alfalfa leaf tissue extracts and two commercial samples of alfalfa (A and B). Myricetin (marked by arrow) was eluted at 25.4 min.

For the ligation reaction, the polymerase-treated DNA (500−1500 ng) was mixed with 5 μL of 10× ligase buffer and 1.25 units of ligase (Life Technologies, Carlsbad, CA). A temperature cycle ligation reaction cycled every 10 s between 10 and 30 °C for 12 h. After the enzyme treatments, the DNA was extracted with phenol/chloroform and subsequently precipitated with 95% ethanol (*10*).

DNA Amplification. The repaired DNA (100−500 ng depending on the level of recovery) was amplified in an MJ Research PTC-100 or PTC-200 thermal cycler using a hot start procedure. For each reaction, 4 μL of 10× X-*Taq* buffer, 4 μL of 2.5 mM dNTP mix, 1 μL of each primer (∼500 ng; 100 pmol), and the repaired DNA were added and brought to a final volume of 40 μL using HPLC-grade water. A hot start mix [8.5 μL of HPLC-grade water, 1 μL 10× X-*Taq* buffer, and 0.5 μL (2.5 units) TaKaRa *Taq* polymerase (TaKaRa] was added both immediately and after 5 min, giving a final volume of 50 μL. Both time points for addition of the hot start mix worked. The primers used for the PCR were from the ITS region of nuclear ribosomal DNA, and the sequence of the primers was the same as the ITS-A and ITS-C universal primers of ITS-1 (*11*): 5′-GGAAGGAGAAGTCGTAACAAGG-3′ and 5′-GCAATTCACACCAAGTATCGC-3′. The PCR fragment is 360 bp long, and the ITS-1 region excluding the flanking rRNA DNA is 237 bp long. We also used the ITS-B universal primer, 5′-CTTTTCCTCCGCTTATTGATATG-3′, which in combination with the ITS-A primer, produced a DNA fragment of 750 bp, which included the 3′-part of the 18S RNA, ITS-1, the 5.8S RNA, ITS-2, and the 5′-part of the 26S RNA (*11*). The conditions for amplification were as follows: a 5-min hold at 96 °C until the TaKaRa polymerase hot start mix was added, then 36 cycles of 94 °C for 15 s, 65 °C for 30 s, and 72 °C for 2 min, followed by 72 °C for 4 min, and finally incubation at 4 °C.

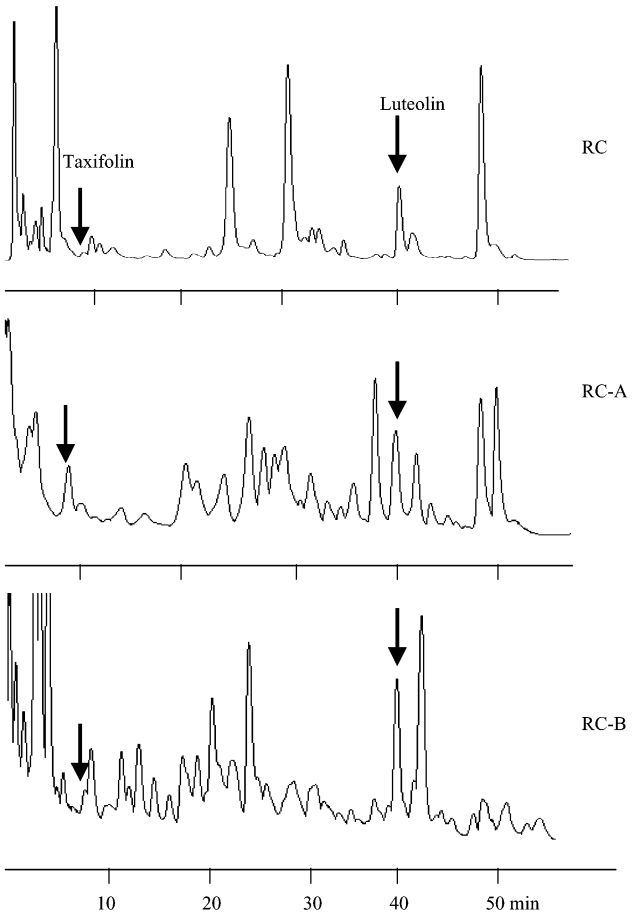

Figure 2. HPLC analysis of red clover leaf and flower tissue extracts and two commercial samples of red clover (A and B). Taxifolin and luteolin (marked by arrows) were eluted at 8.5 and 42 min, respectively.

Electrophoresis and Sequencing. The amplified DNA was subjected to electrophoresis for 1−3 h on a 1% agarose gel, and the bands were subsequently extracted using a Qiagen (Valencia, CA) gel extraction kit. For the various PCR fragments, the DNA was extracted from the gel and prepared for direct sequencing. The DNA sequences were obtained at the UCLA Sequencing Facility in both forward and reverse directions and subsequently analyzed using BLAST (*12*) and Genescan. The complementary DNA strand was also sequenced and used to help identify ambiguous nucleotides and fill in any gaps.

RESULTS

HPLC Analysis. A chromatographic analysis was performed to detect the presence and types of flavonoids in commercial preparations of alfalfa and red clover and was compared to extracts from fresh plant material. Extracts from two commercial samples of alfalfa (**Figure 1**) and red clover (**Figure 2**) are compared to those from freshly extracted alfalfa and red clover leaves. The different flavonoids were identified by retention times using purified samples to calibrate the HPLC experiments. We found that there were significant differences in the HPLC profiles of flavonoids in extracts isolated from fresh tissues and from the two commercial samples using the identical extraction method. For example, the peak that coeluted with myricetin was low in the fresh alfalfa sample and commercial sample A but high in commercial sample B. The flavonoid profiles varied significantly when the different samples were compared (**Figure 1**). Repetitions using different preparations of fresh and commercial extracts gave the same results.

In red clover, taxifolin and luteolin were co-injected and used to identify the peaks in the extracts. The heights of a peak that coeluted with taxifolin, a dihydroflavonol, varied significantly between the fresh and dried samples. Relatively high levels of a compound that coeluted with luteolin were detected in both the fresh and commercial extracts (**Figure 2**). As in the alfalfa analysis, the flavonoid profiles detected by HPLC varied significantly between the fresh material and the dried commercial samples.

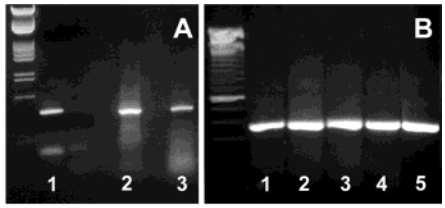

Figure 3. DNA isolated from fresh and dried leaf material after the repair reaction and PCR amplification with the ITS-1 primers: (A) alfalfa (the unmarked lane contains the DNA ladder for lambda DNA restricted with *Eco*RI and *Hin*dIII; the ITS-1 band is just below the 564-bp marker. Lane 1, fresh tissue; lane 2, sample A; lane 3, sample B); (B) red clover (unmarked lanes, DNA ladder of 100 bp increments; the brightest band is 600 bp; lane 1, fresh tissue; lane 2, sample A; lane 3, repetition of sample A; lane 4, sample B; lane 5, repetition of sample B).

DNA Extractions and Amplification. Because the HPLC analysis yielded results that were equivocal in terms of exact plant identification, we utilized DNA-based markers to verify the identity of the plant material within the commercial samples. Using the CTAB method, we were able to extract high-quality DNA from fresh leaves of alfalfa (data not shown). When the same method was applied for isolating DNA from alfalfa leaf powder removed from the commercial capsules, the DNA was found to be degraded and not amplifiable. Slightly better quality, high molecular weight DNA was obtained from the capsular material using the Qiagen DNeasy protocol, but the DNA still could not be amplified (data not shown).

At first, we could not obtain PCR-amplifiable DNA from fresh red clover leaves by using the CTAB method unless the samples were subjected to a phenanthroline cleaning procedure (*13*). By changing to the DNeasy protocol, we could amplify DNA isolated from fresh red clover leaves without the cleaning step. However, none of the cleaning methods, including phenol/chloroform extractions, numerous ethanol precipitations, and phenanthroline cleaning, allowed the amplification of DNA isolated from the powdered plant material contained within gelatin capsules. The DNeasy protocol resulted in more DNA being isolated, but it was sheared and still not amplifiable (data not shown), even though we made a number of modifications to the PCR conditions. For example, the $MgCl_2$ concentration was varied, and increases in the annealing temperature, from 45 to 65 °C in 5 °C increments, were tried; all were unsuccessful. We also utilized four different types of *Taq* polymerase, with and without a hot start. Nevertheless, without the repair reaction, all of these attempts failed.

We then utilized a DNA repair reaction (*5*) and modified it by employing (1) an exonuclease-free Klenow fragment because most Klenow fragments of DNA polymerase exhibit exonuclease activity and (2) longer times for the ligation reaction, 12 h or more versus 60−90 min. In our first attempts, we utilized a repair reaction that incorporated a 24-h fill-in and a 24-h ligation reaction. Later, we found that we could reduce the timing for both the fill-in and ligation reactions to 17 and 12 h, respectively. Subsequent experiments proceeded with the reduced time schedule. The fact that the repair reaction allowed the DNA amplification indicates that the DNA was so highly

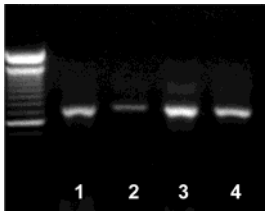

Figure 4. DNA isolated from fresh and dried leaf material of red clover after the repair reaction and PCR amplification with the primers for the entire ITS region: unmarked lanes, DNA ladder of 100 bp increments (the brightest band is 600 bp); lane 1, fresh tissue; lane 2, sample A; lane 3, sample B; lane 4, repetition of sample B.

degraded or fragmented that it minimized the chances of the PCR primers binding to a large enough DNA fragment to amplify a common DNA segment.

PCR Analysis Using the Universal ITS Primers. We initially utilized primers for *rbcL*, a chloroplast DNA sequence frequently used for studies of plant phylogeny, but the length of the resulting PCR fragment precluded its use. Thus, we used primers to the ITS region.

We were able to amplify a PCR fragment of ~360 bp from DNA that had been isolated from either fresh or dried alfalfa tissue (**Figure 3A**) using primers to the ITS-1 region. We sequenced one of the amplified alfalfa DNA bands and found that there was >90% identity and five gaps over 268 bp when the sequence of the DNA isolated from the capsules was compared to the alfalfa sequence in Genbank. Once we determined that the procedure worked for alfalfa, we concentrated our efforts on red clover, which is a more commonly used herbal supplement. **Figure 3B** shows the results of PCR amplification of fresh (lane1) and dried (lanes 2−5) red clover material. The repair reaction resulted in the successful amplification of the DNA isolated from the red clover commercial preparations.

To see whether the DNA was repaired sufficiently to result in a full-length ITS fragment consisting of part of the 18S rRNA, ITS-1, the 5.8S rRNA, ITS-2, and part of the 26S rRNA (*11*), we utilized the ITS-A and ITS-B primers on red clover DNA for producing a PCR fragment of ~750 bp. The PCR fragments from commercial preparations A and B are shown in **Figure 4**.

Sequence Analysis of the Amplified Red Clover DNA. The bands were isolated from the gels, purified, and sent for direct sequencing. The experiments were repeated several times to have multiple bands to check the fidelity of the repair reaction.

For DNA isolated from fresh red clover leaves for two different samples, there was 98 and 99% identity, with no gaps over 237 bp (ITS-1 region only with no flanking sequences), to the comparable *T. pratense* sequence in Genbank. For the repaired, red clover DNA isolated from the sample A capsules, derived from two separate experiments, there was 98% sequence identity to the ITS-1 sequence of *T. pratense* from Genbank. For the repaired, red clover DNA isolated from the sample B capsules, in two separate priming reactions, there was 93 and 97% identity to the ITS-1 region of *T. pratense* from Genbank. Sample B-1, the sample showing 93% identity, has a 21-nucleotide gap at the 3′ end of the sequence (**Figure 5**).

When the sequences for the region consisting of ITS-1, the 5.8 S rRNA, and ITS-2 (613 bp) were analyzed, we found 98 and 99% identity for DNA from samples A and B, respectively, when compared to the *T. pratense* Genbank sequence. The alignments for ITS-1, the 5.8 S rRNA, and ITS-2 for the consensus sequence in Genbank and the two commercial samples are shown in **Figure 6**. All together, these data indicate

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GenBank    1 TTGTCGATGCCTTACATGCAGACCAACACGTGAATCAGTTTGAACACATAGGGTTGGTTT
Fresh-1    1 TTGTCGATGCCTTACATGCAGACCAACACGTGAATCAGTTTGAACACATAGGGCTGGTTT
Fresh-2    1 TTGTCGATGCCTTACATGCAGACCAACACGTGAATCAGTTTGAACACATAGGGCTGGTTT
A-1        1 TTGTCGATGCCTTACATGCAGACCAACACGTGAATCAGTTTGAACACATAGGGCTGGTTT
A-2        1 TTGTCGATGCCTTACATGCAGACCAACACGTGAATCAGTTTGAACACATAGGGCTGGTTT
B-1        1 TTGTCGATGCCTGACATGCAGACCAACACGTGAATCAGTTTGAACACATAGGGCTGGTTT
B-2        1 TTGTCGATGCCTTACATGCAGACCAACACGTGAATCAGTTTGAACACATAGGGCTGGTTT

GenBank   61 GAGGTGTTCAACACCTCGGCTTGCCTCTGGTTCGGAGGATGACGACTTGTGCGTCCTCCT
Fresh-1   61 GAGGTGTTCAACACCTCGGCTTGCCTCTGGTTCGGTGGATGACCACTTGTGCGTCCTCCT
Fresh-2   61 GAGGTGTTCAACACCTCGGCTTGCCTCTGGTTCGGTGGATGACGACTTGTGCGTCCTCCT
A-1       61 GAGGTGTTCAACACCTNGGCTTGCCTCTGGTTCGGTGGATGACGACTTGTGCGTCCTCCT
A-2       61 GAGGTGTTCAACACCTNGGCTTGCCTCTGGTTCGGTGGATGACGACTTGTGCGTCCTCCT
B-1       61 GAGGTGTTCAACACCTTGGCTTGCCTCTAGTTCGGTGGATGACGACTTGAGCCTCCTCTT
B-2       61 GAGGTGTTCAACACCTCGGCTTGCCTCTGGTTCGGTGGATGACGACTTGTGCGTCCTCCT

GenBank  121 CTGGGCCAAAACACAAACCCCGGCGCTGAATGCGTCAAGGAATTTAAAATTTGTTCTGAG
Fresh-1  121 CTGGGCCAAAACACAAACCCCGGCGCTGAATGCGTCAAGGAATTTAAAATTTGTTCTGAG
Fresh-2  121 CTGGGCCAAAACACAAACCCCGGCGCTGAATGCGTCAAGGAATTTAAAATTTGTTCTGAG
A-1      121 CTGGGCCAAAACACAAACCCCGGCGCTGAATGCGTCAAGGAATTTAAAATTTGTTCTGAG
A-2      121 CTGGGCCAAAACACAAACCCCGGCGCTGAATGCGTCAAGGAATTTAAAATTTGTTCTGAG
B-1      121 TTGGGCCAAAACACAAACCCTGGCGCTGAATGCGTCAAGGAATTTAAAATTTGGTCTGAG
B-2      121 NTGGGCCAAAACACAAACCCCGGCGCTGAATGCGTCAAGGAATTTAAAATTTGTTCTGAG

GenBank  181 CGCACCTGCATGCCACCGGAGACGGTTTTCGTGCGGGTTGTGTTCCGACCCTAATAT
Fresh-1  181 CGCACCTGCATGCCACCGGAGACGGTTTTCGTGCGGGTTGTGTTCCGACCCTAATAT
Fresh-2  181 CGCACCTGCATGCCACCGGAGACGGTTTTCGTGCGGGTTGTGTTCCGACCCTAATAT
A-1      181 CGCACCTGCATGCCACCGGAGACGGTTTTCGTGCGGGNTGTGTTCCGACCCTAATAT
A-2      181 CGCACCTGCATGCCACCGGAGACGGTTTTCGTGCGGGTTGTGTTCCGACCCTAATAT
B-1      181 CGCACCTGCATGCCACCGGAGACGGGTTTCGNNCGG--------------------
B-2      181 CGCACCTGCATGCCACCGGAGACGGTTTTCGTGCGGGTTGTGTTCCGACCCTAATAT
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Figure 5. Boxshade diagram comparing the ITS-1 region (237 nuc) of the red clover consensus sequence from Genbank, two preparations of DNA from fresh red clover leaf tissue, and two repetitions of DNA from two different commercial samples (A and B) (N = ambiguous nucleotide and − = a gap). Lack of shading indicates no match, and light shading refers to nucleotide similarity.

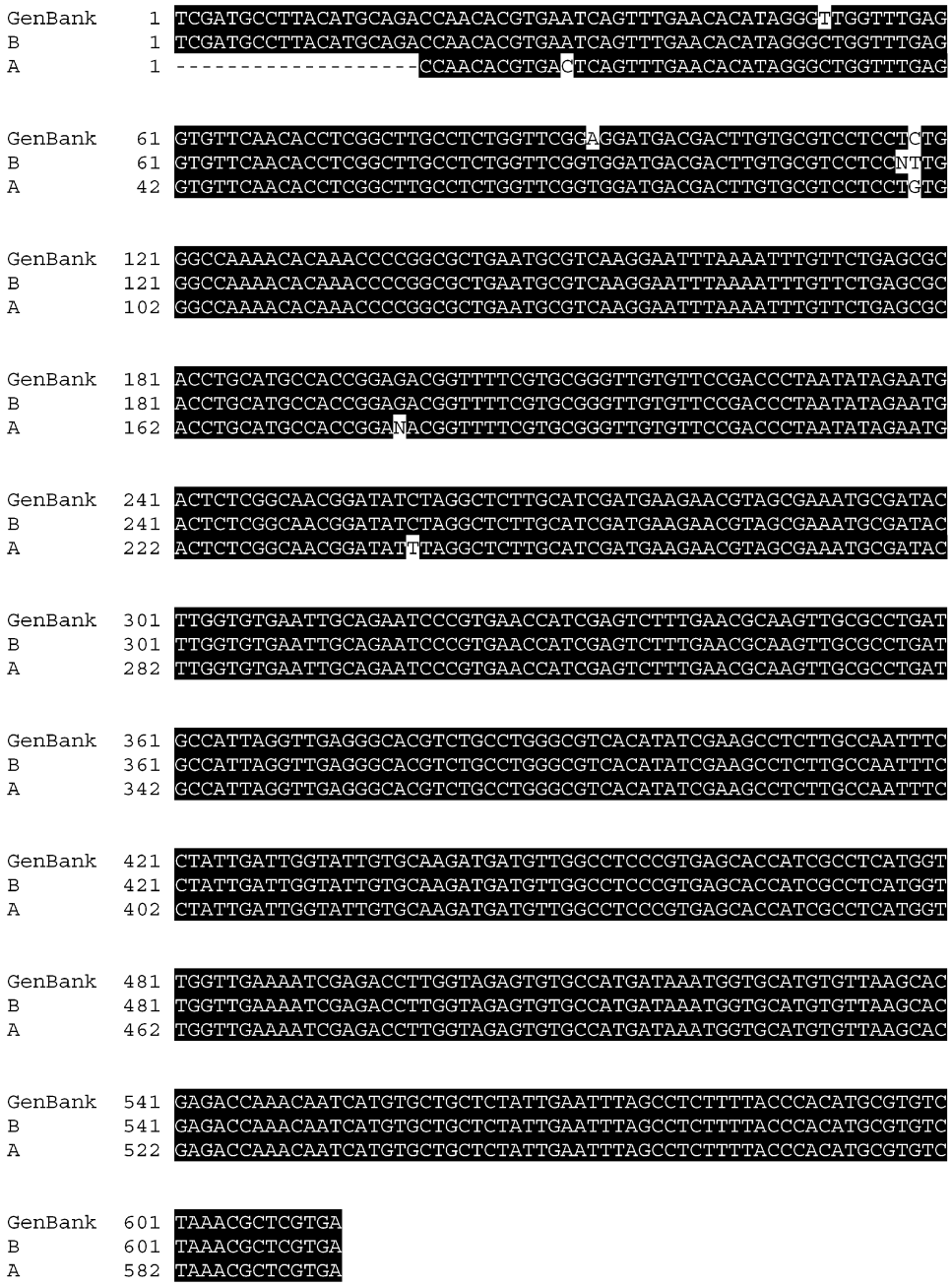

Figure 6. Boxshade diagram comparing the ITS-1, the 5.8 S rRNA, and the ITS-2 region (613 nuc) of the red clover consensus sequence from Genbank, and DNA from two different commercial samples (A and B) (N = ambiguous nucleotide and — = a gap). Lack of shading indicates no match, and light shading refers to nucleotide similarity.

that the powdered plant material contained within the gelatin capsules almost exactly matched the red clover sequences in Genbank.

DISCUSSION

In this study, we report a method for a relatively rapid determination of the identity and hence the potential efficaciousness of herbal supplements, particularly those that have been either ground to a fine powder or improperly dried or stored. The analysis of flavonoid profiles from identically extracted fresh and commercial samples was ambiguous. The differences in the flavonoid profiles of the commercial versus the fresh samples may be caused by the way the various plant materials were prepared, by the different environmental conditions under which the plants were grown, or by the presence of contaminating plant material. For example, treating tissues in water or alcohol for extended periods of time may result in the loss of hydrophilic or hydrophobic compounds. The different drying methods, vacuum versus air-drying, may also alter the HPLC profiles as may differences in growth conditions (*2*, *3*). Because flavonoid profiles can be affected by these factors, we developed a DNA-based assay using PCR to monitor the identity of the plant material stored in gelatin capsules and sold as dietary supplements. PCR amplification of conserved DNA sequences has been used primarily in plant biology for phylogenetic analyses (*14*) and for distinguishing hybrids from parental species (*15*). A distinct advantage of using PCR is that it involves the use of minute quantities of DNA, which is of value especially when the dried plant material is refractory to the isolation of amplifiable DNA or when only small amounts of

DNA can be obtained. The procedure involves the isolation of DNA using the DNeasy reagents, followed by a repair reaction, before the PCR. The repair reaction was developed by Pusch et al. (*5*) to amplify ancient DNA (aDNA), which is usually damaged due to DNase activity or mechanical and physical actions and hence not amplifiable. The repair reaction fills in the nicks and breaks in the small, fragmented, duplex DNA strands, thereby maximizing the chances for the complementary PCR primers to amplify a common DNA segment.

We originally used primers to the ITS-1 region because it is smaller than either the entire ITS region or ITS-2. Initially, we were not certain as to how efficient the repair reaction would be; later we determined that we could amplify the entire ITS region. The ITS region of nuclear ribosome DNA is one of the more widely used DNA sequences in angiosperm molecular systematics (see ref *16*). ITS-1 and ITS-2 separate the genes encoding the 18S, 5.8S, and 26S ribosomal subunits in plants and are generally used to uncover phylogenetic trends between closely related organisms, such as species within a genus, because they are much less conserved than the actual coding regions of the rRNA genes (*17*). The noncoding gene region, ITS-1, which separates the 18S rRNA gene from the 5.8S rRNA gene, was primarily used for this study not only because it is small (<250 bp) but also because there are numerous sequences (>5000) in Genbank.

Two recent studies have described the identification of medicinal plants using PCR analysis of isolated DNA. Lau et al. (*18*) used the ITS-2 region to differentiate medical *Dendrobium* species from one other and also from nonorchids and adulterants. Mihalov et al. (*19*) used oligonucleotides based on Genbank accession entries for *Panax ginseng* and *Panax quinquefolius* to the entire ITS region as well as primers to the chloroplast *rbcL* gene to identify commercial preparations of ginseng. In contrast to our results, Mihalov et al. (*19*) were able to amplify DNA from commercial preparations of ginseng without utilizing a repair reaction. The reason for this difference in results may relate to the quality of the starting material or to other differences such as tissue source or method of DNA extraction. We were unable to amplify DNA from the powdered plant material following conventional DNA isolation procedures unless the repair reaction was performed. After the repair was accomplished, we could determine with certainty that the commercial preparations were either alfalfa or red clover and that they appeared to be unadulterated with other plant material. The ITS-1 regions of alfalfa from the fresh material and from two herbal samples were almost identical, as were the comparable ITS-1, 5.8 SRNA, and ITS-2 regions from the samples of dried and fresh red clover.

The PCR-based identification procedure described herein allows us to authenticate and monitor, in a few easy steps, the identity and integrity of material sold over-the-counter as botanicals in dietary supplements, even material that has been ground to a fine powder or improperly dried or stored. This approach should be applicable to any plant species that is utilized as an herbal supplement. Moreover, it should also enable us in the future to use such DNA "fingerprints" obtained by PCR to develop high-throughput methods for rapid identification of botanicals in dietary supplements.

ABBREVIATIONS USED

BLAST, basic local alignment search tool; bp, base pairs; CTAB, hexadecyltrimethylammonium bromide; HPLC, high-performance liquid chromatography; ITS, internal transcribed spacer; PCR, Polymerase Chain Reaction.

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